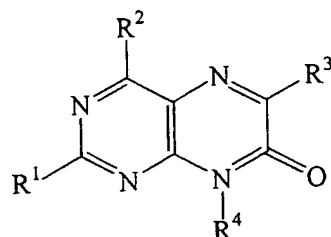


WHAT IS CLAIMED IS:

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a2

1. A compound of the formula:



wherein:

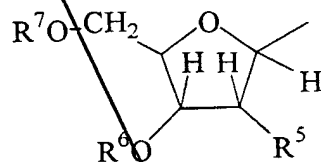
R¹ is a member selected from the group consisting of hydrogen and optionally substituted C₁-C₆-alkyl;

R² is a member selected from the group consisting of amino and mono- or di-substituted amino wherein the substituent is a protecting group;

R³ is optionally substituted C₁-C₆ alkyl;

R⁴ is a member selected from the group consisting of hydrogen and L;

L is of the formula



wherein:

R⁵ is a member selected from the group consisting of hydrogen, hydroxyl, and substituted hydroxyl wherein the substituent is a protecting group;

R⁶ is a member selected from the group consisting of hydrogen, phosphoramidite, an H-phosphonate, a methyl phosphonate, a phosphorothioate, a phosphotriester, a hemisuccinate, a hemisuccinate covalently bound to a solid support, a dicyclohexylcarbodiimide, and a dicyclohexylcarbodiimide covalently bound to a solid support, a hydroxyalkyl, and a hydroxyalkyl covalently bound to a solid support; and

R⁷ is a member selected from the group consisting of hydrogen, a phosphate, a triphosphate, and a protecting group;

with the proviso that R¹ and R⁴ are not simultaneously L.

2. A compound in accordance with claim 1, wherein R¹ is hydrogen;

R² is a member selected from the group consisting of amino, mono-, and di-substituted amino wherein the substituents are members selected from the group consisting of benzoyl, isobutyryl, phthaloyl, di-n-butylaminomethylidene,

5 dimethylaminomethylidene, p-nitrophenylethoxycarbonyl and
6 dimethylaminomethylenamino;

7 R^4 is L;

8 R^5 is a member selected from the group consisting of hydrogen, hydroxyl,
9 hydroxyl substituted with a member selected from the group consisting of trityl,
10 monomethoxytrityl, dimethoxytrityl, tetrahydropyran-1-yl, 4-methoxytetrahydropyran-4-
11 yl, 1-(2-chloro-4-methyl)phenyl-4-methoxypiperidin-4-yl, t-butyldimethylsilyl, p-
12 nitrophenylethylsulfonyl, tetrahydropyranyl, 4-methoxytetrahydropyranyl, 2-nitrobenzyl,
13 9-phenylxanthen-9-yl and p-nitrophenylethyl;

14 R^6 is a member selected from the group consisting of consisting of
15 hydrogen, phosphoramidite, H-phosphonate, hemisuccinate, and hemisuccinate
16 covalently bound to a solid support; and

17 R^7 is a member selected from the group consisting of hydrogen, trityl,
18 monomethoxytrityl, dimethoxytrityl, phthaloyl, di-n-butylaminomethylene,
19 dimethylaminomethylidene and triphosphate.

1 3. A compound in accordance with claim 2, wherein R^2 is a member
2 selected from the group consisting of amino and an amino group mono-substituted by a
3 protecting group selected from the group consisting of di-n-butylaminomethylidene, p-
4 nitrophenylethoxycarbonyl, and dimethylaminomethylenamino;

5 R^5 is a member selected from the group consisting of hydrogen, hydroxyl
6 and hydroxyl substituted with a member selected from the group consisting of
7 dimethoxytrityl, tetrahydropyran-1-yl, t-butyldimethylsilyl, 2-nitrobenzyl, and p-
8 nitrophenylethylsulfonyl;

9 R^6 is a member selected from the group consisting of hydrogen, β -
10 cyanoethyl-N-diisopropyl phosphoramidite and a hemisuccinate covalently bound to
11 controlled pore glass; and

12 R^7 is a member selected from the group consisting of dimethoxytrityl, di-
13 n-butylaminomethylene, and dimethylaminomethylidene.

1 4. A compound in accordance with claim 2, wherein R^2 is a member
2 selected from the group consisting of amino and an amino group mono-substituted by a
3 protecting group selected from the group consisting of di-n-butylaminomethylidene, p-
4 nitrophenylethoxycarbonyl, and dimethylaminomethylenamino;

5 R⁵ is a member selected from the group consisting of hydrogen and
6 hydroxyl substituted with a member selected from the group consisting of
7 dimethoxytrityl, tetrahydropyran-1-yl, t-butyldimethylsilyl, 2-nitrobenzyl, and p-
8 nitrophenylethyl;

9 R⁶ is a member selected from the group consisting of hydrogen and β -
10 cyanoethyl-N-diisopropyl phosphoramidite; and

11 R⁷ is a member selected from the group consisting of hydrogen and
12 dimethoxytrityl.

1 5. A compound in accordance with claim 2, wherein R² is a member
2 selected from the group consisting of amino and dimethylaminomethylenamino;

3 R³ is methyl;

4 R⁵ is hydrogen;

5 R⁶ is β -cyanoethyl-N-diisopropyl phosphoramidite; and

6 R⁷ is dimethoxytrityl.

1 6. A compound in accordance with claim 2, wherein R² is amino;

2 R³ is methyl;

3 R⁵ is hydrogen;

4 R⁶ is β -cyanoethyl-N-diisopropyl phosphoramidite; and

5 R⁷ is dimethoxytrityl.

1 7. A compound in accordance with claim 2, wherein R² is amino;

2 R³ is methyl;

3 R⁵ is hydrogen;

4 R⁶ is hydrogen; and

5 R⁷ is hydrogen.

1 8. A compound in accordance claim 2, wherein R² is
2 dimethylaminomethylenamino;

3 R³ is methyl;

4 R⁵ is hydrogen;

5 R⁶ is β -cyanoethyl-N-diisopropyl phosphoramidite; and

6 R⁷ is dimethoxytrityl.

1 9. A compound in accordance with claim 2, wherein R² is amino;

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2 R³ is methyl;
3 R⁵ is hydrogen;
4 R⁶ is β -cyanoethyl-N-diisopropyl phosphoramidite; and
5 R⁷ is a triphosphate.

1 10. A compound in accordance with claim 1, wherein;
2 R¹ is optionally substituted C₁-C₆ alkyl;
3 R² is a member selected from the group consisting of amino, mono-, and
4 di-substituted amino wherein the substituent is a member selected from the group
5 consisting of benzoyl, isobutyryl, phthaloyl, di-n-butylaminomethylidene,
6 dimethylaminomethylidene, p-nitrophenylethoxycarbonyl and
7 dimethylaminomethylenamino;

8 R³ is optionally substituted C₁-C₆ alkyl;

9 R⁴ is L;

10 R⁵ is a member selected from the group consisting of hydrogen, hydroxyl
11 and hydroxyl substituted with a member selected from the group consisting of trityl,
12 monomethoxytrityl, dimethoxytrityl, tetrahydropyran-1-yl, 4-methoxytetrahydropyran-4-
13 yl, 1-(2-chloro-4-methyl)phenyl-4-methoxypiperidin-4-yl, t-butyl dimethylsilyl, p-
14 nitrophenylethylsulfonyl, tetrahydropyranyl, 4-methoxytetrahydropyranyl, 2-nitrobenzyl,
15 9-phenylxanthene-9-yl and p-nitrophenylethyl;

16 R⁶ is a member selected from the group consisting of hydrogen, H-
17 phosphonate, phosphoramidite, hemisuccinate, and hemisuccinate covalently bound to a
18 solid support; and

19 R⁷ is a member selected from the group consisting of hydrogen, trityl,
20 monomethoxytrityl, dimethoxytrityl, phthaloyl, di-n-butylaminomethylene, and
21 dimethylaminomethylidene.

1 11. A compound in accordance with claim 10 wherein R¹ is methyl;

2 R² is a member selected from the group consisting of amino and an amino
3 group mono-substituted by a protecting group selected from the group consisting of di-n-
4 butylaminomethylidene, p-nitrophenylethoxycarbonyl, and
5 dimethylaminomethylenamino;

6 R³ is methyl;

7 R⁵ is a member selected from the group consisting of hydrogen, hydroxyl
8 and hydroxyl substituted with a member selected from the group consisting of
9 dimethoxytrityl, tetrahydropyran-1-yl, t-butyldimethylsilyl, 2-nitrobenzyl, and p-
10 nitrophenylethylsulfonyl;

11 R⁶ is a member selected from the group consisting of hydrogen, β -
12 cyanoethyl-N-diisopropyl phosphoramidite and a hemisuccinate covalently bound to
13 controlled pore glass; and

14 R⁷ is a member selected from the group consisting of dimethoxytrityl, di-
15 n-butylaminomethylene, and dimethylaminomethylidene.

1 12. A compound in accordance claim 10, wherein R¹ is methyl; R² is a
2 member selected from the group consisting of amino and an amino group mono-
3 substituted by a protecting group selected from the group consisting of di-n-
4 butylaminomethylidene, p-nitrophenylethoxycarbonyl, and
5 dimethylaminomethylenamino;

6 R⁵ is a member selected from the group consisting of hydrogen and
7 hydroxyl substituted with a member selected from the group consisting of
8 dimethoxytrityl, tetrahydropyran-1-yl, t-butyldimethylsilyl, 2-nitrobenzyl, and p-
9 nitrophenylethylsulfonyl;

10 R⁶ is a member selected from the group consisting of consisting of
11 hydrogen and β -cyanoethyl-N-diisopropyl phosphoramidite; and

12 R⁷ is a member selected from the group consisting of hydrogen and
13 dimethoxytrityl.

1 13. A compound in accordance with claim 10, wherein R¹ is methyl;
2 R² is a member selected from the group consisting of amino and
3 dimethylaminomethylenamino;

4 R³ is methyl;

5 R⁵ is hydrogen;

6 R⁶ is β -cyanoethyl-N-diisopropyl phosphoramidite; and

7 R⁷ is dimethoxytrityl.

1 14. A compound in accordance with claim 10, wherein R¹ is methyl;
2 R² is amino;

3 R³ is methyl;

R⁵ is hydrogen;

R⁶ is β -cyanoethyl-N-diisopropyl phosphoramidite; and

R⁷ is dimethoxytrityl.

15. A compound in accordance with claim 10, wherein R¹ is methyl;

R² is amino;

R³ is methyl;

R⁵ is hydrogen;

R⁶ is hydrogen; and

R⁷ is hydrogen.

16. A compound in accordance with claim 10, wherein R¹ is methyl; is dimethylaminomethylenamino;

R³ is methyl;

R⁵ is hydrogen;

R⁶ is β -cyanoethyl-N-diisopropyl phosphoramidite; and

R⁷ is dimethoxytrityl.

17. A compound in accordance with claim 10, wherein R¹ is methyl;

R² is amino;

R³ is methyl;

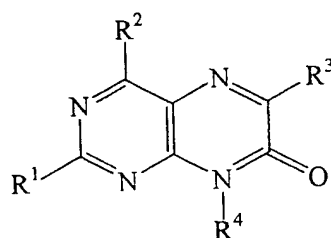
R⁵ is hydrogen;

R⁶ is β -cyanoethyl-N-diisopropyl phosphoramidite; and

R⁷ is a triphosphate.

18. An oligonucleotide comprising one or more nucleotide monomers,

said monomers having the formula



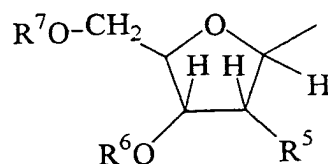
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4 wherein:

5 R¹ is a member selected from the group consisting of hydrogen and

6 optionally substituted C₁-C₆-alkyl;

- 7 R^2 is a member selected from the group consisting of amino and mono- or
 8 di-substituted amino wherein the substituent is a protecting group;
 9 R^3 is optional substituted C_1 - C_6 alkyl;
 10 R^4 is L;
 11 L is of the formula



- 12 wherein:
 13 R^5 is a member selected from the group consisting of hydrogen and
 14 hydroxyl;
 15 R^6 is a member selected from the group consisting of hydrogen, a
 16 phosphate, a phosphate covalently attached to a nucleotide, a phosphate covalently
 17 attached to a nucleoside; a hemisuccinate covalently bound to a solid support, a
 18 dicyclohexylcarbodiimide covalently bound to a solid support, and a hydroxyalkyl
 19 covalently bound to a solid support; and
 20 R^7 is a member selected from the group consisting of hydrogen, a
 21 phosphate, a phosphate covalently attached to a nucleotide and a phosphate covalently
 22 attached to a nucleoside;
 23 with the proviso that R^1 and R^4 are not simultaneously L.
 24

- 1 19. An oligonucleotide in accordance with claim 18, wherein:
 2 R^1 is hydrogen;
 3 R^2 is amino;
 4 R^3 is methyl;
 5 R^5 is hydrogen and hydroxyl;
 6 R^6 is hydrogen; and
 7 R^7 is a phosphate.

- 1 20. An oligonucleotide in accordance with claim 19, wherein:
 2 R^5 is hydrogen.

- 1 21. An oligonucleotide in accordance with claim 19 wherein:

2 R⁵ is hydroxyl.

1 22. An oligonucleotide in accordance with claim 18, wherein:

2 R¹ is optionally substituted C₁-C₆-alkyl;

3 R² is amino;

4 R³ is methyl;

5 R⁵ is hydrogen and hydroxyl;

6 R⁶ is hydrogen; and

7 R⁷ is a phosphate.

1 23. An oligonucleotide in accordance with claim 22, wherein

2 R¹ is methyl and

3 R⁵ is hydrogen.

1 24. An oligonucleotide in accordance with claim 22, wherein

2 R¹ is methyl and

3 R⁵ is hydroxyl.

1 25. An oligonucleotide in accordance with claim 18, wherein said

2 nucleotide monomers are at the 3' end of said oligonucleotide.

1 26. An oligonucleotide in accordance with claim 18, wherein said

2 nucleotide monomers are at the 5' end of said oligonucleotide.

1 27. An oligonucleotide in accordance with claim 18, wherein said

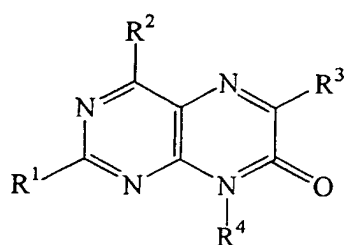
2 nucleotide monomers are surrounded by 1 to 10 pyrimidine monomers.

1 28. An oligonucleotide in accordance with claim 18, wherein said

2 oligonucleotide is a member selected from the group consisting of SEQ ID:1, SEQ ID:2,
3 SEQ ID:3, SEQ ID:4, SEQ ID:5, SEQ ID:6, SEQ ID:7, SEQ ID:8, SEQ ID:9, SEQ ID:10,
4 SEQ ID:11, SEQ ID:12, SEQ ID:13, SEQ ID:14, SEQ ID:15, SEQ ID:16, SEQ ID:17,
5 SEQ ID:18, SEQ ID:19, SEQ ID:20, SEQ ID:21 and SEQ ID:22.

1 29. A method of detecting the presence, absence, or quantity of a target
2 nucleic acid, said method comprising the steps of:

3 a) contacting said target nucleic acid with a nucleic acid probe wherein said
4 nucleic acid probe comprises compound of the formula:



wherein:

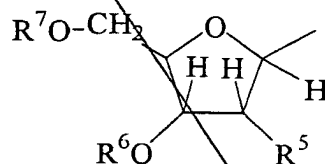
R^1 is a member selected from the group consisting of hydrogen and optionally substituted C_1 - C_6 -alkyl;

R^2 is a member selected from the group consisting of amino and mono- or di-substituted amino wherein the substituent is a protecting group;

R^3 is optionally substituted C_1 - C_6 alkyl;

R^4 is L;

L is of the formula



wherein:

R^5 is a member selected from the group consisting of hydrogen and hydroxyl;

R^6 is a member selected from the group consisting of hydrogen, phosphoramidite, an H-phosphonate, a methyl phosphonate, a phosphorothioate, a phosphotriester, a hemisuccinate, a hemisuccinate covalently bound to a solid support, a dicyclohexylcarbodiimide, and a dicyclohexylcarbodiimide covalently bound to a solid support; and

R^7 is phosphate;

with the proviso that R^1 and R^4 are not simultaneously L, located in said probe such that, when said probe hybridizes to said target nucleic acid said compound is in a loop that does not participate in complementary base pairing with a nucleotide of said target nucleic acid; and

b) detecting the fluorescence produced by said fluorescent nucleotide when said probe forms a hybrid duplex with said target nucleic acid.

30. A method of claim 29, wherein said loop ranges in length from about 1 to about 100 nucleotides when said probe hybridizes to said target nucleic acid.

- 1 31. A method of claim 29, wherein said loop is an insertion in said
2 nucleic acid probe which is otherwise complementary to said target nucleic acid or to a
3 contiguous subsequence of said target nucleic acid.
- 1 32. A method of claim 31, wherein said insertion is three nucleotides in
2 length and comprises two nucleotides each adjacent to said compound.
- 1 33. A method of claim 32, wherein at least one nucleotide adjacent to
2 said compound is a purine.
- 1 34. A method of claim 33, wherein at least one nucleotide adjacent to
2 said compound is an adenosine.
- 1 35. A method of claim 32, wherein at least one nucleotide adjacent to
2 said compound is a pyrimidine.
- 1 36. A method of claim 35, wherein at least one nucleotide adjacent to
2 said compound is a cytosine.
- 1 37. A method of claim 34, wherein said compound is bordered by at
2 least two adjacent purines in both the 5' and 3' direction.
- 1 38. A method of claim 37, wherein said adjacent purines are adenosine.
- 1 39. A method of claim 31, wherein said insertion is said compound.
- 1 40. A method of claim 31, wherein said insertion is self-
2 complementary and forms a hairpin wherein said compound is present in the loop of said
3 hairpin and does not participate in complementary base pairing.
- 1 41. A method of claim 29, wherein the nucleotides comprising said
2 loop are selected such that they are not complementary to the corresponding nucleotides
3 of the target nucleic acid when said probe is hybridized to said target nucleic acid and
4 wherein said probe is complementary to at least two non-contiguous subsequences of said
5 target nucleic acid.
- 1 42. A method of claim 29, wherein said fluorescent nucleotide is
2 present in a terminal subsequence of said nucleic acid probe wherein said terminal

3 subsequence does not hybridize to said target nucleic acid when the remainder of said
4 nucleic acid probe hybridizes to said target nucleic acid.

1 43. A method of claim 42, wherein said terminal subsequence forms a
2 terminal hairpin by hybridization with a second subsequence of said probe such that said
3 fluorescent nucleotide is present in a loop of said hairpin and does not participate in
4 complementary base pairing.

1 44. A method of claim 29, wherein said detecting comprises detecting
2 an increase in fluorescence of said fluorescent nucleotide when said probe forms a hybrid
3 duplex with said target nucleic acid.

1 45. A kit for the detection of nucleic acid-nucleic acid interactions
2 comprising a container, said container containing a compound in accordance with claim
3 1, and instructions for use.

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